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(54) Title: CEREAL PRODUCTS HAVING LOW PHYTIC ACID CONTENT

(57) Abstract: An infant cereal product which contains a phytic acid containing protein source but which has reduced phytic acid content. The cereal product is obtained by incubating a mixture including a starch base, a phytic acid containing protein source and a whole grain cereal flour which is an endogeneous phytase source, to reduce the phytic acid content of the mixture. The mixture is then heated to gelatinise the starch base and inactivate the endogeneous phytase. The cereal product has a phytic acid content of less than 1µmol/g of phytic acid.

Cereal Products Having Low Phytic Acid Content

Field of the invention

This invention relates to cereal products foods which contain mixtures of cereals and legumes and which are substantially free of phytic acid. The cereal products are particularly suitable for use as weaning foods. The invention also relates to a process for producing the cereal products.

Background to the invention

After an infant reaches the age of about 4 to 6 months, solid or semi-solid foods having high nutrient density are needed to meet the requirements of rapid growth. These solid or semi-solids foods are termed weaning foods. Further, the bioavailability of minerals and trace elements from weaning foods should be high to cover the infant's needs during rapid growth.

Weaning foods usually take the form of infant cereal products which contain one or more cereals and a protein source such as milk or legumes. Cereals and legumes contain phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate), a natural component and phosphorous storage form of the plant. Phytic acid binds strongly to minerals and trace elements such as Fe^{3+}, Zn^{2+}, Cu^{2+}, and Mg^{2+} at the pH of the digestive tract. This makes the minerals and trace elements unavailable for absorption.

In cereals and legumes, phytic acid occurs mostly in the form of inositol-6-phosphate, which can be degraded to lower inostiol-phosphates or inositol during food processing by the enzyme phytase. This might improve mineral and trace element absorption. The enzymatic degradation of phytic acid may be done by using commercial phytase preparations; often obtained from micro-organisms such as *Aspergillus niger*.

It has recently been suggested that certain cereals such as rye, triticale, wheat and buckwheat may be potential sources of phytase (Egli, I., Davidsson, L., and Hurrell, R.F.; *Phytase Activity of Cereals, Pseudocereals and Legumes*, Poster, 1997). However, there is no suggestion that these phytase sources may be used in the production of weaning foods. Further, simpler procedures are needed to degrade phytic acid in the raw materials making up infant cereal products.

Therefore it is an object of this invention provide a cereal product which contain mixtures of cereals and legumes and which is substantially free of phytic acid.

Summary of the invention

Accordingly, in one aspect, this invention provides an infant cereal product which comprises a gelatinised starch base containing a phytic acid containing protein source and a whole grain cereal flour which is an endogeneous phytase source.

The infant cereal product is preferably a weaning food which is in ready to eat form.

In another aspect, this invention provides an infant cereal product which contains a phytic acid containing protein source and has reduced phytic acid content, the product obtainable by a process which comprises:-

incubating a mixture including a starch base, a phytic acid containing protein source and a whole grain cereal or whole grain cereal flour which is an endogenous phytase source to reduce the phytic acid content of the mixture; and

heating the mixture to gelatinise the starch base and inactivate the endogeneous phytase.

In a further aspect, this invention provides a process for preparing an infant cereal product which has reduced phytic acid content, the process comprising:-

incubating a mixture including a starch base, a phytic acid containing protein source and a whole grain cereal flour which is an endogeneous phytase source to reduce the phytic acid content of the mixture; and

heating the mixture to gelatinise the starch base and inactivate the endogeneous phytase.

In another aspect, this invention provides the use of a whole grain cereal flour which is an endogeneous phytase source for reducing the phytic acid content of infant cereal product which contains a phytic acid containing protein source.

Detailed description of the preferred embodiments

Embodiments of the invention are now described by way of example only. This invention is based upon the discovery that certain whole grain cereals are

endogeneous phytase sources which may, by their inclusion into the infant cereal product, degrade the phytic acid in the infant cereal product. The degradation may be complete.

The whole grain cereal may be any suitable cereal which is an endogeneous phytase source. Cereals which are endogeneous phytase sources may be readily determined by combining, in solution, a milled sample of the cereal with a phytic acid substrate and then measuring the liberation of inorganic phosphate. For example the whole grain cereal may be barley, buckwheat, wheat, triticale or rye. Preferably the whole grain cereal is in flour form.

The whole grain cereal preferably provides about 1% to about 20% by dry weight of the infant cereal product; for example about 5% to about 15% by weight. The exact amount used will depend upon the phytic acid content of the ingredients of the infant cereal product and the activity of the phytase. However, the amount may be readily determined for any particular application.

The starch base may be provided by any suitable starch source. Particularly suitable are starch containing cereal flours such as wheat flour, rice flour, corn flour, millet flour, oat flour, and sorghum flour. Alternative, the starch base may be in the form of potato starch or potato. Also, mixtures of these sources may be used.

The starch source preferably provides about 40% to about 80% by dry weight of the infant cereal product; for example about 50% to about 75% by weight.

The phytic acid containing protein source may be a legume or oil seed which provides a suitable source of protein. For example, the legume may be soy, mung bean, cow pea, chickpea, pea and lentil and the oil seed may be cotton seed. The legume or oil seed is preferably in the form of a flour or protein concentrate. Especially preferred is soy flour.

The legume or oil seed preferably provides about 10% to about 60% by dry weight of the infant cereal product; for example about 15% to about 30% by weight. The exact amount will be selected to provide the desired protein content; for example about 10% to about 20% by weight protein.

The infant cereal product may also contain, if desired, other suitable sources of protein such as non fat milk solids, whey and casein.

Further, infant cereal product may also contain a suitable lipid source. Suitable lipid sources include milk fat, corn oil, olive oil, sunflower oil, safflower oil, rape seed oil, coconut fat, palm oil, palm kernel oil, and fish oil.

To improve the organoleptic properties of the infant cereal product, the infant cereal product may contain sweeteners and suitable flavouring agents. Suitable sweeteners include sucrose, maltose, lactose, maltodextrin, and corn syrup solids. Further, artificial sweeteners such as saccharin, cyclamates, acetosulfame, sucralose, and L-aspartyl based sweeteners such as aspartame may be included if desired and permitted.

The infant cereal product is preferably fortified with minerals and vitamins to meet the needs of the infants. Suitable minerals include calcium, phosphorous, iodine, iron, magnesium, copper, zinc, manganese, chloride, potassium, sodium, selenium, chromium, and molybdenum. Suitable vitamins include vitamin A, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin E, vitamin K, vitamin C, vitamin D, folic acid, niacin, biotin, pantothenic acid, choline, and biotin. The levels of the vitamins and minerals may be selected as desired and in accordance with prevailing regulations for infant cereal products.

The infant cereal product may optionally contain other substances which may have a beneficial effect such as fibres, lactoferrin, nucleotides, nucleosides, and the like.

The infant cereal product may be produced by any suitable technique. An important step, however, is the incubation of the legume or oil seed flour with the whole grain cereal flour to enable the endogeneous phytase to degrade the phytic acid. If the starch base contains phytic acid, the starch base is preferably also incubated with the whole grain cereal.

For optimum degradation of the phytic acid, the whole grain cereal flour, the legume or oil seed flour, and the starch base are slurried in an aqueous liquid; preferably water. The temperature and pH of the slurry are preferably adjusted to values at which the phytase is optimally active. The temperature is preferably in the range of about 35°C to about 60°C; for example about 50°C to about 55°C. The pH is preferably in the range of about 4.5 to about 6.0; for example about 5.0 to about 5.6. If necessary, a suitable acid such as citric acid, tartaric acid, malic acid, hydrochloric acid, or the like, may be added to adjust the pH.

The slurry is incubated for a time sufficiently long to degrade the phytic acid to an acceptable level; preferably completely. This time will vary depending upon the phytic acid content, type and amount of legume or oil seed, the type and amount of whole grain cereal, the dry matter content of the slurry, the temperature, and the pH used. However, in any situation, the optimum time may

be rapidly determined by simple experiment. Usually however, a time in the range of 60 minutes to about 200 minutes will be suitable.

The level to which the phytic acid is depleted is a matter of choice but is preferably as low as possible. For example, levels less than about 1 μmol/g of cereal product are preferred; especially less than about 0.1 μmol/g.

After the slurry has been incubated, the slurry should be heat treated to gelatinise the starch base, inactivate any enzymes, and pasteurise the slurry. This may be done in any suitable manner.

For example, the slurry may be heated by the direct injection of steam or by using a suitable heat exchanger such as a scraped surface heat exchanger. Ordinarily, the temperature of the slurry is raised to above about 120°C for at least 2 seconds. Preferably the temperature is raised to above about 130°C for at least about 5 seconds. Thereafter, the slurry may be cooled; for example by flash cooling. The slurry may then be filled into containers, if a liquid cereal product is desired, or may be dried by means of spray-drying or roller drying. Roller drying is preferred since a flaked cereal product is obtained.

As another example, the slurry may be heat treated in an extrusion cooker. To improve the textural properties of the final product, a compressed gas is preferably injected into the extrusion cooker prior to the extrusion die. A suitable process is described in US patent 5,385,746; the disclosure of which is incorporated by reference.

If desired, the slurry may be subjected to further enzymatic treatment prior to being transformed into its final form. For example, the slurry may be incubated with an enzyme such as an amylase to break down starch in the starch base. This is preferably done after the slurry has been heat treated a first time to gelatinise the starch base and inactivate any enzymes. By breaking down the starch base, the viscosity of the weaning food reconstituted from the final product is more stable. Also, sugars are produced which improves the taste of the cereal product. The wettability of the cereal product may also be improved.

The infant cereal product may be used as a weaning food for infants by reconstituting it with water or milk or other suitable liquid. Because the cereal product has reduced levels of or contains no phytic acid, the cereal product is able to provide a nutritionally density food having high bioavailability of minerals and trace elements. Therefore the cereal product is ideally suited to weaning infants.

Example 1

A dry blend of 28 kg of wheat flour (type 550), 8 kg of soy flour and 4 kg of whole grain rye flour is prepared in a mixer. The dry blend has a protein content of about 15% by weight. An amount of 100 litres of water at 50°C is added to the dry blend under mixing. Citric acid is then added to adjust the pH to about 5.5. The resulting slurry is then incubated under mixing for 3 hours at 50°C. Samples of the slurry are taken at the start (time 0) and 20 minute intervals thereafter.

The incubated slurry is heated to 135°C for about 30 seconds using steam injection to inactivate any enzymes, pasteurise the slurry and gelatinise starch. The heated slurry is then flash cooled to a temperature of about 70°C. The cooled slurry is dried to flakes on a roller drier.

Immediately upon taking of any sample of the slurry, hydrochloric acid is added to the sample to terminate any enzymatic reactions and to extract any phytic acid present. The inositol phosphates (IP3, IP4, IP5, IP6) in the extract are determined according to the procedure of Sandberg and Ahderinne (*J. Food Sci.*; 54, 1986, 547-550) using HPLC analysis with refractive index detection. In a similar fashion, a sample of the flakes is extracted with hydrochloric acid and analysed for the different inositol phosphates.

The total inositol phosphate content of the samples is as follows:-

Sample	**Total Inositol Phosphates / μmol/g**
time = 0 minutes	5.31
time = 20 minutes	0.83
time = 40 minutes	0.16
time = 60 minutes	0.15
time = 80 minutes	0.12
time = 100 minutes	not detectable
Flakes	not detectable

The results indicate that the cereal product is virtually free of phytic acid.

Example 2

A dry blend of 28 kg of wheat flour (type 550), 8 kg of soy flour and 4 kg of whole grain wheat flour is prepared in a mixer. The dry blend has a protein content of about 15% by weight. An amount of 100 litres of water at 50°C is added to the dry blend under mixing. Citric acid is then added to adjust the pH to about 5.1. The resulting slurry is then incubated for 3 hours at 50°C under mixing and then processed to flakes as described in example 1.

Further, samples of the slurry and flakes are taken and analysed for inositol phosphate content as described in example 1. The total inositol phosphate content of the samples is as follows:-

Sample	Total Inositol Phosphates / µmol/g
time = 0 minutes	6.08
time = 20 minutes	2.96
time = 40 minutes	0.19
time = 60 minutes	0.12
time = 80 minutes	traces.
time = 100 minutes	traces.
Flakes	not detectable

The results indicate that the cereal product is substantially devoid of phytic acid.

Example 3

A study in humans was carried out. The aim of this study was to measure the influence of complete phytic acid degradation in an infant cereal product based on wheat and soybean on zinc and copper absorption in adults. The negative influence of phytic acid on iron absorption in adults and infants is well established in the literature. In contrast, the effect of phytic acid on zinc absorption has been contradictory and there is very limited information of the influence of phytic acid on copper absorption. *In vitro* studies suggest a strong interaction between phytic acid and copper, although until now this has not been confirmed in humans.

An infant cereal product with no phytic acid (< 0.1 μmol/g), produced with the technique described above (example 2), was compared with an infant cereal product containing the native phytic acid content (circa 6 μmol/g). Each study subject (seven premenopausal women and two men) was given the infant cereal product with no phytic acid and the infant cereal product with the native phytic acid content in a cross-over design, enabling each subject to act as her/his own control. Zinc and copper were labeled extrinsically with stable isotopes (^{70}Zn and ^{65}Cu) and apparent zinc and copper absorption was measured based on a fecal monitoring technique. Isotopic analysis was performed by thermal ionization mass spectrometry. Apparent fractional zinc and copper absorption was calculated based on fecal excretion of zinc and copper isotopic labels during six day fecal collection periods, indicated by brilliant blue. The completeness of the fecal collection was verified by the excretion of a non-absorbable fecal marker (dysprosium).

The mean apparent fractional zinc absorption was significantly higher ($p = 0.005$) from the infant cereal product with no phytic acid (34.6%, range 26.1-51.7%), than from the infant cereal product with the native phytic acid content (22.8%, range 10.4-32.3%). No significant difference ($p = 0.167$) was found for the mean apparent fractional copper absorption between the infant cereal product with no phytic acid (19.7%, range 11.0-26.3%) and the infant cereal product with the native phytic acid content (23.7%, range 15.5-42.3%).

The results clearly demonstrated a beneficial effect on zinc absorption after dephytinization of infant cereal products.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without

departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

Claims

1. An infant cereal product which comprises a gelatinised starch base containing a phytic acid containing protein source and a whole grain cereal or a whole grain cereal flour which is an endogenous phytase source; the cereal product containing less than 1 µmol/g of phytic acid.

2. An infant cereal product which contains a phytic acid containing protein source and has reduced phytic acid content, the product obtainable by a process which comprises:-

incubating a mixture including a starch base, a phytic acid containing protein source and a whole grain cereal flour which is an endogeneous phytase source to reduce the phytic acid content of the mixture; and

heating the mixture to gelatinise the starch base and inactivate the endogeneous phytase.

3. An infant cereal according to claim 2 in which the mixture is incubated at a pH of about 4.5 to about 6.0.

4. An infant cereal product according to any of claims 1 to 3 which contains less than 0.1 µmol/g of phytic acid.

5. An infant cereal product according to any of claims 1 to 4 in which the whole grain cereal flour is selected from one or more of barley flour, buckwheat flour, wheat flour, triticale flour and rye flour.

6. An infant cereal product according to any of claims 1 to 5 in which the phytic acid containing protein source is soy.

7. An infant cereal product according to any of claims 1 to 6 in which the whole grain cereal flour provides about 1% to about 20% by dry weight of the infant cereal product.

8. An infant cereal product according to any of claims 1 to 6 which has a protein content of about 10% to about 20% by weight.

9. A process for preparing an infant cereal product which has reduced phytic acid content, the process comprising:-

incubating a mixture including a starch base, a phytic acid containing protein source and a whole grain cereal flour which is an endogeneous phytase source to reduce the phytic acid content of the mixture; and

heating the mixture to gelatinise the starch base and inactivate the endogeneous phytase.

10. The use of a whole grain cereal flour which is an endogeneous phytase source for reducing the phytic acid content of infant cereal product which contains a phytic acid containing protein source.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/105 A23L1/164 A23L1/211

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	X.S. ZHU: "Preparation of a Low-Phytate Feed Mixture and Bioavailability of its Phosphorus to Chicks" ANIMAL FEED SCIENCE AND TECHNOLOGY., vol. 27, no. 4, 1990, pages 341-351, XP002120644 AMSTERDAM., NL page 341, paragraph 1 page 342, paragraph 2 page 343, paragraph 4 -page 344, paragraph 1	1-10
X	US 5 904 942 A (MIYAKE TOSHIO ET AL) 18 May 1999 (1999-05-18) the whole document	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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Inter   onal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1997 LI JIAN RONG: "Enzymic Removal of Antinutritional Factors in Soybean" retrieved from STN Database accession no. 127:134966 XP002120646 abstract & ZHONGGUO LIANGYOU XUEBAO, vol. 12, no. 2, 1997, pages 15-20, ---	1-10
A	WO 98 11788 A (LAHDEN POLTTIMO OY;SEMPER AB) 26 March 1998 (1998-03-26) the whole document ---	1-10
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Inte onal Application No
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